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SHORT COMMUNICATION

SCREENING COWPEA AND SOYBEAN CULTIVARS FOR RESISTANCE TO ANTHRACNOSE AND BROWN BLOTCH DISEASES USING PHYTOTOXIC METABOLITES

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ABSTRACT

Toxic metabolites of *Colletotrichum lindemuthianum* and *C. truncatum* produced in culture induced necrotic lesions on cowpea and soybean leaves, stems and pods. Bioassay of the culture filtrates of the two pathogens using 14 cultivars each of cowpea and soybean indicated that host plants reacted differentially by producing different sizes of lesions. The 14 cultivars were grouped as susceptible, moderately resistant and resistant, and groupings were similar to those reported from field tests using *C. lindemuthianum* and *C. truncatum* on cowpea and soybean cultivars.

Key Words*: *Colletotrichum lindemuthianum*, *C. truncatum*, *Glycine max*, toxins, *Vigna unguiculata

RÉSUMÉ

Des métabolites toxiques de culture de *Colletotrichum lindemuthianum* et *C. truncatum* ont provoqué des lésions de nécrose sur les feuilles, tiges et gousses du niébé et du soja. Lorsque les filtrats de culture des deux pathogènes ont été chacun testés sur des cultivars de niébé et de soja (14 pour chaque test), il á été remarqué que les plantes hôtes ont différemment réagi exhibant des lésions de différentes tailles. Les 14 cultivars ont été alors groupés en cultivars sensibles, moyennement résistants et resistants. Catte classification s'est avérée identique à celle obtenue à partir des tests de terrain utilisant *C. lindemuthianum* et *C. truncatum* sur des variétés de niébé et de soja.

Mot Clés: Colletotrichum lindemuthianum*, *C. truncatum*, cire de glycine, toxines, *Vigna unguiculata

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) and soybean (*Glycine max* L.) are high in protein and other essential nutrients which greatly improve or otherwise blend unbalanced diets (IITA, 1985; Singh and Rachie, 1985). They are also highly compatible as companions with a wide range of food and fibre crops. These two crops, however, suffer disease damage which have greatly reduced

their production in Nigeria. In a bid to effectively manage cowpea and soybean diseases, several control measures have been adopted (IAR, 1984; IITA, 1985). However, the most effective approach to managing diseases is the selection and breeding for disease resistant varieties (Nwankiti *et al.*, 1987).

Phytotoxic metabolites of pathogens play significant roles in pathogenesis. In this study, phytotoxic metabolites produced by *Colletotrichum lindemuthianum* and *C. truncatum* in culture were used to screen cowpea and soybean cultivars for resistance to anthracnose and brown blotch diseases.

MATERIALS AND METHODS

Cowpea and soybean leaves, stems and pods showing symptoms of anthracnose and brown blotch diseases were collected from demonstration plots at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The samples were washed in running tap water, and cut into small pieces. These were then surface sterilized using 10% sodium hypochlorite, and rinsed in five successive changes of sterile distilled water. The infected portions were plated out in Potato Dextrose Agar (PDA) and incubated for 6 days at 26°C under 12-hr photoperiod. The causative pathogens were identified by microscopic examination as well as comparing the isolates with standards which were obtained from the Pathology Laboratory of IITA, Ibadan.

Toxin Production. Erlenmeyer Flasks (250 ml), containing 100 ml of sterilized Czapek Dox medium, were inoculated with three fungal mycelial disks (5 mm diameter) cut from the margin of 5-day-old cultures of the pathogens. The flasks were shaken at 100 rpm for 21 days at 26°C. Mycelial mats were removed by passing the cultures through four layers of cheese cloth. The filtrates were then reduced to 1/10th of their original volumes in a rotary evaporator at 40°C.

Bioassay. Leaves of cowpea (cv.IT 1784E-16) and soybean (cv.SAMSOY) known to be susceptible to *Colletotrichum* spp. were raised in the greenhouse. After 8 weeks, the leaves were excised with sterile razor blades at the petioles and covered with wet sanitary cotton. Ten microlitres of the metabolites of each pathogen were individually spot inoculated on one-half of each leaflet. On the other half, spots of uninoculated Czapek Dox medium were placed as control. A sterile office pin was used to prick through the centre of each spot. The treated leaves were placed in sterile moist Petri dishes and incubated at 26°C, and disease reaction observed after 24 hours. Three leaves per plant were inoculated for each crop type and these were replicated three times.

Screening for disease resistance. Fourteen cultivars of cowpea and soybean were screened with the toxic metabolites of *C. lindemuthianum* and *C. truncatum*. Screening for resistance to anthracnose and brown blotch in both cowpea and soybean was carried out using leaf, stem, and pod puncture bioassay techniques. Eight week - old cowpea and soybean leaves, stems, and pods from the greenhouse showing no symptoms of infection were excised from the shoots and brought to the laboratory. They were rinsed in running tap water, dipped in 10% sodium hypochlorite for 30 seconds and then rinsed in five changes of sterile distilled water. The plant parts were individually placed in sterile Petri dishes lined with moistened sterile filter paper. Subsequently, three 10 µL droplets of the concentrated phytotoxic metabolites were placed on these plant parts which were then punctured with sterile needles at the centre of each droplet. Ten leaves, stems, and pods were inoculated per cultivar of each crop type and these were replicated three times.

Test plants were rated for reaction using a modified lesion diameter scale of Sudi and Podhardizky (1959) as follows: < 7.0 mm = highly resistant; 7.1–11.0 mm = resistant; 11.1–15.0 mm = moderately susceptible; and ≥ 15.1 mm = susceptible. The diameter of necrotic lesions induced on these crops and their parts were recorded and transformed to $\log_e$. The data were then subjected to analysis of variance and the Least Significant Difference (LSD) was used to seperate the means at $P = 0.05$.

RESULTS

The metabolites of C. *truncatum* and *C. lindemuthianum* in culture were phytotoxic to cowpea and soybean, and the symptoms produced

TABLE 1. Response of 14 cowpea cultivars to *in vitro* inoculation with phytotoxic metabolites of *Colletotrichum truncatum* and *C. lindemuthianum*[a]

Cultivar	Mean size of the necrotic lesion							
	C. truncatum				*C. lindemuthianum*			
	Leaves	Stems	Pods	X[b]	Leaves	Stems	Pods	X[b]
IT282^{E}-16	20.2	15.8	14.7	18.2 a	20.7	16.3	14.6	17.2 a
TVU3236	21.7	16.0	13.4	17.6 a	21.6	15.2	14.1	16.9 ab
TW 300	20.4	15.4	14.6	17.2 ab	17.5	15.9	14.9	16.1 bc
IT82^{E}-32	19.2	15.4	14.4	16.9 bc	15.5	14.2	12.9	14.2 g
TVU-1994	18.3	16.2	14.9	16.6 bc	18.3	15.2	13.9	15.8 cde
TVU-1990	18.2	15.5	14.4	16.2 cd	18.5	14.1	12.8	15.2 def
IT81^{D}-1137	16.2	15.7	13.8	16.0 cd	16.9	16.9	14.1	15.3 def
IFE-BROWN	16.5	16.4	14.7	15.9 d	18.6	15.4	13.6	15.9 d
IT82^{D}-60	16.4	15.9	14.7	15.8 d	18.5	16.3	13.9	16.3 bc
848-2245-4	17.6	15.7	13.7	15.7 d	16.2	14.4	14.1	14.9 fg
TVU-3232	15.4	14.5	13.4	14.6 e	15.2	14.2	13.3	14.3 g
IT82^{D}-699	13.9	13.7	11.1	13.0 f	17.2	15.9	14.4	15.8 cde
IT81^{D}-773	13.5	13.0	11.5	12.8 f	15.5	14.8	13.7	14.7 fg
IT82^{D}	11.6	12.1	11.2	11.6 g	15.7	14.2	13.3	14.4 g

[a]Each value is a mean of 5 replicates (5 measurements per replicate) and is a transformation from the $\log_e$ of the original value.
[b]Values followed by the same letters are not significantly different ($P < 0.05$) by the Least Significant Differences test.

by the metabolites were similar to those induced by the pathogens themselves. *C. truncatum* induced necrotic lesions on IT81D-994, IT82D-699, TVU-3232 and IT81D-773 of between 11.6 and 14.6 mm diameters (Table 1). These cultivars were considered to be moderately susceptible to the toxic metabolite. The other 10 cultivars were susceptible.

TABLE 2. Responses of 14 soybean cultivars to *in vitro* inoculation with phytotoxic metabolites of *Colletotrichum truncatum*[a]

Cultivars	Mean necrotic lesion size (mm)		
	Leaves	Pod	X[b]
536^{D}-20	23.6	19.4	21.5 a
932-2^{E}	23.6	18.9	21.3 a
TGX923^{D}	22.9	18.8	20.9 ab
SAMSOY	25.6	18.3	20.3 ab
1614-1^{E}	21.9	19.3	20.6 ab
TGM-337	20.7	18.9	19.8 bc
TGM-298	20.3	2.6	19.4 bc
1-851^{D}	19.2	16.7	18.2 sd
TGM-297-2	18.1	17.2	17.7 de
TGM-623	18.3	15.3	16.8 def
TGX-536-02^{D}	17.8	15.2	16.5 ef
TGM-705	15.2	15.0	15.4 f
TGM-236	10.6	10.1	10.3 g
PI-17144	3.5	7.2	5.3 h

[a]Each value is a mean of 5 replicates (5 measurements per replicate) and is a transformation from the $\log_e$ of the original value.
[b]Values followed by the same letters are not significantly different ($P < 0.05$) by the Least Significant Difference test.

All the 14 cowpea cultivars displayed susceptible necrotic lesions to the metabolite of *C. lindemuthianum* (Table 1). Five cultivars IT82E-32, TVU 3232, IT82D-994, IT81o-773 and 848-2246-4 had necrotic lesions of between 14.00 - 14.90 mm (moderately susceptible). The most susceptible cultivars were TVU-300, IT82E-60, TVU-3236 and IT82E-16 while TVU-1990, IT81D-137, TVU-1994, IT82D-699, and ife brown showed intermediate reactions.

Soybean cultivars PI-17144 and TGM 236 had mean necrotic lesions of 5.3 mm and 10.3 mm, respectively. These two cultivars were considered very resistant to soybean anthracnose, while the remaining 12 cultivars were susceptible (Table 2).

DISCUSSION

Colletotrichum species are known to produce phytotoxic metabolites in culture (Masatoshi *et al.*, 1976; 1978; Walker and Templeton, 1978). Toxic metabolites were produced by the two species tested in this study. Cowpea cultivars

IT82D-994, IT845-22464 and IT82E-32 are reportedly susceptible to anthracnose and brown blotch diseases (Adebitan, 1991). This corresponds well with our results. Similarly, in field and screenhouse studies, Ife brown and IT82D-60 were susceptible to *C. lindemuthianum* and *C. truncatum* (Emechebe and Shoyinka, 1985). The reactions of these two cultivars to the toxic metabolites of the two pathogens also showed that they were susceptible.

The soybean cultivars PI-17144 and TGM-236 are resistant to anthracnose (IITA, 1987). These cultivars also demonstrated similar reactions to toxic metabolites of the pathogens, while the reactions of some of the other 12 cultivars to the toxic metabolites of C. *trunctum* corresponded to the greenhouse and field screening experiments (IITA, 1987).

From these results therefore it appears that screening for disease resistance with toxic metabolites obtained from C. *trunctum* and *C. lindemuthianum* is practicable. Also, with the use of these metabolites, rapid screening of a large population of the crops within a short period of time (24 hours) is possible (Hell and Weber, 1986).

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